

AMENDMENT TO THE CLAIMS:

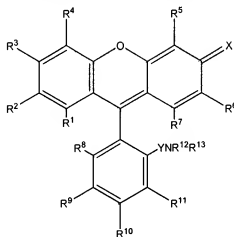
This listing of claims will replace all prior versions, and listings of claims in the application:

LISTING OF CLAIMS:

The list of currently pending claims is presented below.

WHAT IS CLAIMED IS:

1. (Original) A xanthene dye having the formula:



in which

R¹, R², R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ and R¹¹ are independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, halogen, H, NO₂, CN and C(Z¹)R¹⁴, NR¹⁵R¹⁶ and Z²R¹⁶;

R³ is selected from Z²R¹⁶ and NR¹⁵R¹⁶

wherein

Z^1 is a member selected from O, S and NH;

Z^2 is a member selected from O and S;

R^{15} is a member selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl;

R^{16} is selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, $C(Z^3)R^{17}$, and a nitrogen-containing reactive group comprising R^{15} and R^{16} , together with the nitrogen to which they are attached, wherein said reactive group is a member selected from $-NHNH_2$, $-N=C=S$ and $-N=C=O$

wherein

Z^3 is a member selected from O, S and NH;

R^{17} is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OR^{18} , and $NR^{19}R^{20}$

wherein

R^{18} is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and $C(O)R^{21}$

wherein

R^{21} is substituted or unsubstituted alkyl or substituted or unsubstituted heteroalkyl;

R^{19} and R^{20} are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl

Y is a member selected from $C(O)$ and $S(O)_2$;

X is a member selected from (NR²²R²³) and (O)

wherein

R²² and R²³ are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

R¹² and R¹³ are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl, with the proviso that at least one of R¹² or R¹³ comprises a member selected from a bond to a carrier molecule, a bond to a linker bound to a carrier molecule, a bond to a solid support, a bond to a linker attached to a solid support, a bond to a fluorescence quencher, a bond to a linker to a fluorescence quencher and an oxygen-containing reactive group, and further with the proviso that when R¹² and R¹³, together with the nitrogen to which they are attached form a piperazine ring said oxygen-containing reactive group is a phosphoramidite and said bond to a carrier molecule is other than a bond to a peptide.

2. (Original) The xanthene dye according to claim 1, wherein R³ is R¹⁵R¹⁶N; and X is NR²³R²⁴, wherein R¹⁵, R¹⁶, R²³ and R²⁴ are members independently selected from H, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

3. (Original) The xanthene dye according to claim 1, wherein at least one of R⁸, R⁹, R¹⁰ and R¹¹ is a halogen.

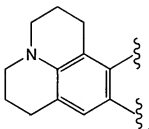
4. (Original) The xanthene dye according to claim 1, wherein R⁹ and R¹⁰ are halogen.

5. (Original) The xanthene dye according to claim 3, wherein R³ is OR¹⁶; and X is O.

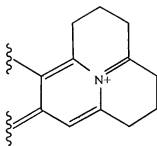
6. (Original) The xanthene dye according to claim 5, wherein R² and R⁶ are halogen.

7. (Original) The xanthene dye according to claim 5, wherein R^2 and R^6 are independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

8. (Original) The xanthene dye according to claim 1, wherein R^3 is $NR^{15}R^{16}$ and R^2 , R^4 and R^{15} and R^{16} , together with the nitrogen atom to which they are bound, are fused with the phenyl moiety to which $NR^{15}R^{16}$, R^2 and R^4 are bound, forming a substituted or unsubstituted ring system having formula:



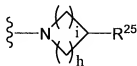
9. (Original) The xanthene dye according to claim 1, wherein X is $NR^{22}R^{23}$ and R^5 , R^6 and R^{22} and R^{23} , together with the nitrogen atom to which they are bound, are fused with the unsaturated 6-member ring to which $NR^{22}R^{23}$, R^5 and R^6 are bound, forming a substituted or unsubstituted ring system having the formula:



10. (Original) The xanthene dye according to claim 1, wherein said oxygen-containing reactive functional group is a member selected from hydroxyl and activated derivatives thereof, phosphoramidite, and carboxylic acid and activated derivatives thereof.

11. (Original) The xanthene dye according to claim 1, wherein R^{12} and R^{13} , together with the nitrogen to which they are bound are joined to form a ring system.

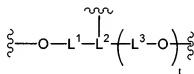
12. (Original) The xanthene dye according to claim 11, wherein NR¹²R¹³ has the formula:



wherein

h and i are members independently selected from integers such that the sum (h + i) is from 4-8; and
R²⁵ is a reactive functional group.

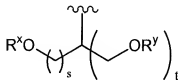
13. (Original) The xanthene dye according to claim 1, wherein R¹² comprises a moiety having the formula:



wherein

L^1 , L^2 and L^3 are members independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and t is 0 or 1.

14. (Original) The xanthene dye according to claim 13, said moiety having the formula:



wherein

R^x and R^y are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, a hydroxyl-

protecting group, a phosphate moiety, a phosphodiester moiety, a phosphorus-containing internucleotide bridge of a nucleic acid, a solid support, a carrier molecule and $-\text{OP}(\text{O})(\text{OR}^9)(\text{N}(\text{R}^p\text{R}^q)_2)$

wherein

R^9 , R^p and R^q are members independently selected from H, substituted or unsubstituted C_1 - C_6 alkyl and substituted or unsubstituted C_1 - C_6 heteroalkyl; and
 s is an integer from 1 to 20.

15. (Original) The xanthene dye according to claim 14, wherein R^9 is $\text{CH}_2\text{CH}_2\text{CN}$.

16. (Original) The xanthene dye according to claim 14, wherein at least one of R^x and R^y comprises a moiety having the formula:

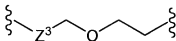


wherein

L^4 is a member selected from a bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

R^z is a member selected from a reactive functional group, solid support, a nucleic acid, a saccharide and a peptide.

17. (Original) The xanthene dye according to claim 16, wherein L^4 comprises a moiety having the formula:



wherein Z^3 is a member selected from CH_2 and $\text{C}=\text{O}$.

18. (Original) The xanthene dye according to claim 1, wherein said carrier molecule further comprises a quencher moiety.

1 **19.** (Original) The xanthene dye according to claim 18, wherein said xanthene dye
2 and said quencher comprise a donor-acceptor energy transfer pair.

1 **20.** (Original) The xanthene dye according to claim 18, wherein said quencher has
2 substantially no native fluorescence.

1 **21.** (Original) The xanthene dye according to claim 20, wherein said quencher
2 comprises at least three residues selected from aryl, substituted aryl, heteroaryl, substituted
3 heteroaryl and combinations thereof, wherein at least two of said residues are covalently linked
4 via an exocyclic diazo bond.

1 **22.** (Original) The xanthene dye according to claim 1, wherein said xanthene dye is
2 attached to a nucleic acid at a position which is a member selected from the 3'-terminus, the 5'-
3 terminus, a nucleobase, and a phosphorus-containing internucleotide bridge of said nucleic acid.

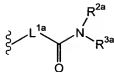
1 **23.** (Original) The xanthene dye according to claim 18, wherein said nucleic acid is a
2 probe which is a member selected from molecular beacons, scorpion probes, sunrise probes,
3 conformationally assisted probes and TaqMan™ probes.

1 **24.** (Original) The xanthene dye according to claim 1, wherein said carrier molecule
2 is a peptide comprising a cleavage recognition site for an enzyme.

1 **25.** (Original) The xanthene dye according to claim 24, wherein said peptide
2 comprises a cleavage recognition site for a protease.

1 **26.** (Original) The xanthene dye according to claim 24, wherein said cleavage
2 recognition site is for an enzyme selected from trypsin, enterokinase, HIV-1 protease,
3 prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase,
4 cytomegalovirus assemblin, leishmanolysin, β -secretase for amyloid precursor protein, thrombin,
5 renin, angiotensin-converting enzyme, cathepsin-D and a kininogenase.

1 **27.** (Original) The xanthene dye according to claim 1, in which R¹² has the formula:



wherein

L^{1a} is a member selected from substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl groups; and

R^{2a} and R^{3a} are members independently selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl, and R^2 and R^3 , together with the nitrogen to which they are attached, are optionally joined to form a ring which is a member selected from substituted or unsubstituted C_5 - C_7 cycloalkyl and substituted or unsubstituted 5-7-membered heterocycloalkyl.

28. (Original) The xanthene dye according to claim 27, in which L^{1a} does not comprise a member selected from a carboxylic acid and a carboxylic acid ester.

29. (Original) A method for determining whether a sample contains an enzyme, said method comprising:

(a) contacting said sample with a peptide construct comprising:

- i) a xanthene dye according to claim 1;
- ii) a quencher; and
- iii) a cleavage recognition site for said enzyme,

wherein said peptide is in a conformation allowing donor-acceptor energy transfer between said fluorophore and said quencher when said fluorophore is excited;

(b) exciting said xanthene dye; and

(c) determining a fluorescence property of said sample, wherein the presence of said enzyme in said sample results in a change in said fluorescence property.

1 **30.** (Original) A method for determining whether a compound alters an activity of an
2 enzyme, said method comprising:

3 (a) contacting a sample comprising said enzyme and said compound with a peptide
4 construct comprising:

5 i) a xanthene dye according to claim 1;

6 ii) a quencher; and

7 iii) a cleavage recognition site for said enzyme,

8 wherein said peptide is in a conformation allowing donor-acceptor energy transfer
9 between said xanthene dye and said quencher when said xanthene dye is
10 excited;

11 (b) exciting said xanthene dye; and

12 (c) determining a fluorescence property of said sample, wherein said activity of said
13 enzyme in said sample results in a change in said fluorescence property.

1 **31.** (Original) A method for detecting a nucleic acid target sequence, said method
2 comprising:

3 (a) contacting said target sequence with a detector oligonucleotide comprising a target
4 binding sequence, said detector oligonucleotide having linked thereto,

5 i) a xanthene dye according to claim 1; and

6 ii) a quencher,

7 wherein said detector nucleic acid is in a conformation allowing donor-acceptor
8 energy transfer between said xanthene dye and said quencher when said
9 xanthene dye is excited;

10 (b) hybridizing said target binding sequence to said single-stranded target sequence,
11 thereby altering said conformation of said detector oligonucleotide, causing a change
12 in a fluorescence parameter; and

(c) detecting said change in said fluorescence parameter, thereby detecting said nucleic acid target sequence.

32. (Original) The method according to claim **31**, wherein said complementary strand is synthesized in a target amplification reaction.

33. (Original) The method according to claim **31**, wherein said complementary strand is synthesized by extension of the target sequence using said detector oligonucleotide as a template.

34. (Currently Amended) The method according to claim **31**, wherein said fluorescence parameter is detected in [[-]]real-time.

35. (Original) A method for detecting amplification of a target sequence comprising, in an amplification reaction:

(a) hybridizing to said target sequence a detector oligonucleotide comprising a single-stranded target binding sequence and an intramolecularly associated secondary structure 5' to said target binding sequence, wherein at least a portion of said detector sequence is a single stranded tail which is available for hybridization to said target sequence, said detector oligonucleotide having linked thereto,

i) a xanthene dye according to claim **1**; and

ii) a quencher,

wherein said detector nucleic acid is in a conformation allowing donor-acceptor energy transfer between said xanthene dye and said quencher when said xanthene dye is excited;

(b) extending said hybridized detector oligonucleotide on said target sequence with a polymerase to produce a detector oligonucleotide extension product and separating said detector oligonucleotide extension product from said target sequence;

- (c) hybridizing a primer to said detector oligonucleotide extension product and extending the primer with said polymerase, thereby linearizing said intramolecularly associated secondary structure and producing a change in a fluorescence parameter; and
- (d) detecting said change in said fluorescence parameter, thereby detecting said target sequence.

36. (Original) The method according to claim 35, wherein said target sequence is amplified by a method selected from Strand Displacement Amplification, Polymerase Chain reaction, Self Sustained Sequence Replication, Transcription Mediated Amplification, and Nucleic Acid Sequence Based Amplification.

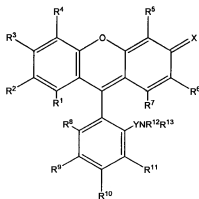
37. (Original) The method according to claim 35, wherein said secondary structure further comprises a partially or entirely single-stranded restriction endonuclease site.

38. (Original) The method according to claim 35, wherein a change in fluorescence intensity is detected.

39. (Original) The method according to claim 38, wherein said change in fluorescence intensity is detected in real-time.

40. (Original) The method according to claim 35, wherein said intramolecularly associated secondary structure comprises a portion of said target binding sequence.

41. (Original) A method of preparing a conjugate between a nucleic acid and a xanthene dye having the formula:



in which

$R^1, R^2, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}$ and R^{11} are independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl, halogen, H, NO_2 , CN and $C(Z^1)R^{14}$, $NR^{15}R^{16}$ and Z^2R^{16} ;

R^3 is selected from Z^2R^{16} and $NR^{15}R^{16}$

wherein

Z^1 is a member selected from O, S and NH;

Z^2 is a member selected from O and S;

R^{15} is a member selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl;

R^{16} is selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, $C(Z^3)R^{17}$, and a nitrogen-containing reactive group comprising R^{15} and R^{16} , together with the nitrogen to which they are attached, wherein said reactive group is a member selected from $-NHNH_2$, $-N=C=S$ and $-N=C=O$

wherein

Z^3 is a member selected from O, S and NH;

R^{17} is a member selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, OR^{18} , and $NR^{19}R^{20}$

wherein

R^{18} is a member selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and $C(O)R^{21}$

wherein

R²¹ is substituted or unsubstituted alkyl or substituted or
unsubstituted heteroalkyl;

R¹⁹ and R²⁰ are members independently selected from H,
substituted or unsubstituted alkyl and substituted or
unsubstituted heteroalkyl

Y is a member selected from C(O) and S(O)₂;

X is a member selected from (NR²²R²³) and (O)

wherein

R²² and R²³ are members independently selected from H, substituted or
unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

R¹² and R¹³ are members independently selected from substituted or unsubstituted alkyl,
substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted
or unsubstituted aryl and substituted or unsubstituted heteroaryl, with the proviso that at least one
of R¹² or R¹³ comprises said nucleic acid,

said method comprising:

(a) contacting a precursor of said conjugate comprising nucleic acid protecting groups
with a mixture of amine and alcohol, thereby removing said protecting groups.